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PANOS D. CALDIS
AND
GEORGE H. COONS

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ACHROMATIC VARIATIONS IN PATHOGENIC FUNGI

PANOS D. CALDIS
College of Agriculture
University of California

AND

GEORGE H. COONS
Michigan State College

FOR many years the development of white tufts of mycelium had been noticed in presumably single-spore pure cultures of fungi. When bits of this white growth were by chance included in the inoculum, it was observed that the white tufts led to the development of white sectors in the cultures. In addition, gradual loss of the power of spore production was a common occurrence with cultures maintained in the laboratory for a long period as stock cultures.

Our attention was focused on this aberrant behavior by the experiences with cultures of *Phoma apiicola* Kleb. in a study of a celery disease begun by one of us (9) and continued by C. W. Bennett (1, 2) of the Department of Botany, Michigan State College. *Phoma apiicola* had been isolated and its pathogenicity to celery definitely shown. A culture was obtained from the International Institute for Pure Cultures and this by comparison cultures and by inoculations seemed to be identical with the American isolation. Work on the celery root rot was interrupted by the war and it was found by Mr. Bennett on his return that the stock cultures had lost color and were completely avirulent. Fresh isolations from diseased material were normal in behavior and of the same color as the early isolations. From the new isolations it was possible occasionally to pick sterile clumps of mycelium and to obtain gray or white forms which apparently had lost virulence and power of spore production.

This work, done with single-spore cultures of a fungus in which the sexual stage was not a factor, brought up many questions as to the proper interpretation of the phenomenon.

Since other cultures in the laboratory, more adaptable to culture tests, showed the same tendency, these have been used in an attempt to throw light upon the problem and to allow us to decide whether these variant forms were mutants, environmental modifications or sterile mycelium which, affected by conditions of long culture, needed special methods for rejuvenation.

METHODS

The following cultures known to give rise to these aberrant forms were chosen for intensive study: *Sphaeropsis malorum* Pk. (Mich. Exp. Sta., No. 125); *Colletotrichum lindemuthianum* (Sacc. and Magn.) Br. and Cav. (Strains I and II); *Cladosporium fulvum* Cke.; *Septoria apii* Chester (freshly isolated).

Although the parent strains named above were progenies of presumably single-spore isolations, that is, isolation (from plates) of growths apparently from one spore, in order to make sure that only single-spore cultures were used in these experiments plates were poured by the ordinary dilution method and very dilute, well-shaken, suspensions of spores in corn meal agar were used. As soon as a well-isolated spore was found to have germinated — usually on the second day — its location in the plate was marked by an ink dot and the agar lying over the dot was scooped out with a flattened, sterile platinum needle. The agar containing the germinating spore was placed on a sterile slide and carefully examined under the high power of the microscope to make sure that only a single spore had been removed from the plate. This requirement once satisfied, the piece of agar containing the spore was again placed on a corn meal agar slant and allowed to grow. In more than twenty such plantings made by this method, there were but few spores that did not grow; none were found to have been contaminated by bacteria or fungi in the process of examination or transfer.

In this way single-spore cultures of the organisms named were obtained. All work subsequently to be reported was done with

these tested isolations. The media used were the standard sorts as well as certain synthetic solutions as indicated in the footnote on page 195. The chemicals used were Baker's analyzed and the glassware, except culture tubes and preparation dishes, was either Pyrex or Jena. All glass culture dishes were cleaned by immersing over night in cleaning solution followed by four rinsings in tap water and one rinsing in distilled water. The water used was distilled water which had been redistilled in a block tin still or in special cases (pH experiments) in a Jena glass still.

TESTS ON LABORATORY MEDIA

Septoria apii

Six single-spore isolations were made on oat meal agar and transferred to corn meal agar, prune juice agar, synthetic medium No. 2 (see note on page 195), carrot plugs and corn meal. The organism grew normally upon all these media for a week, forming a black dense growth with numerous pycnidia scattered on the surface of the medium. Ten days after inoculation a white tuft was found in Isolation 5 (corn meal), forming a striking contrast with the normal black growth (Plate VIII, Fig. 1). Attempts were made to transfer bits of this white mycelium to Richards' solution, oat meal agar and corn meal, but it invariably produced a jet black growth in the new culture medium.

The same strain gave a second white tuft in a culture on prune juice agar. The white tuft transferred to synthetic medium No. 2 gave a black growth, apparently normal. Isolations 1 and 2 behaved in a similar way. Still another isolation developed "white islands" on oat meal agar and synthetic solution No. 2, but grew normally on being transferred.

Sphaeropsis malorum

This culture, which had been in stock for a long time, had presented some difficulties in securing single-spore strains because of the absence of pycnidia. On being grown on Coons' synthetic liquid medium it sporulated abundantly and two single-spore isolations were obtained. These when grown on the standard

media produced luxuriant growth. White tufts appeared on corn meal agar, but vagrant threads of these on being transferred to synthetic medium No. 2 gave rise to dark gray growth with several black pycnidia. No further work was done with this organism.

Colletotrichum lindemuthianum, Strain I

Two single-spore isolations were made. These were grown on the standard media. White tufts appeared on the corn meal and oat meal agar cultures, but transfers of these to corn meal flasks and oat meal agar slants gave black, apparently normal, cultures. Other attempts described later failed to establish a white strain.

Colletotrichum lindemuthianum, Strain II

This fungus was known to be more variable than the preceding one. Five single-spore isolations were made and the

FIG. 2. Cultures of white form of *Colletotrichum lindemuthianum* (*a*), the parent form of *Cladosporium fulvum* (*b*), and the variant form of *C. fulvum* (*c*)

organism was grown on standard media. Many white islands appeared, especially on corn meal and on oat meal agar, and these variations proved to be remarkably constant. Isolation

5 on corn meal produced white tufts of mycelium twenty days after inoculation. Bits of the white mycelium were transferred to oat meal agar, synthetic agar No. 2, prune juice agar, corn meal agar, corn meal; and from these, fifteen subsequent transfers were made throughout the summer on a variety of media. The fungus produced only a white compact mat with very little aërial mycelium. This variant was used in our later studies (see Fig. 2).

Cladosporium fulvum

Four single spore isolations were secured as described. When grown for twenty days in corn meal, Isolation 4 showed a small white island arising from the center of a thick mass of small, normal, olive-green clumps (Plate VIII, Fig. 1). An attempt to pick off the colorless hyphae and grow them on corn meal and oat meal agar failed, presumably because spores were taken along with the white mycelium. On a second attempt the white hyphae were successfully transferred from a culture of the parent strain to prune juice agar, synthetic medium No. 2, corn meal agar and oat meal agar. The laboratory media used, besides those to be discussed later, were Coons' synthetic agar, corn meal, steamed rice, raspberry leaf agar, standard nutrient agar, Czapek synthetic agar and lima bean infusion agar.

Besides Isolation 4, the other single-spore isolation cultures developed white patches at different times and in many cases isolations were secured. Table I presents an account of the appearance of these variations in the different strains and on the different media used. It is evident that the forms occur repeatedly and upon widely varying nutrient media. The different white forms from the various single-spore cultures appeared identical.

It should be noticed that these so-called white forms are not perfectly white, but have a pink or lilaceous tinge in the hyphae. Makemson (20) and Spangler (28) have described the growth of *Cladosporium fulvum* on the host and on the different culture media, giving measurements and drawings of the mycelium, conidiophores and conidia. The parent strain used in these experiments is from the original isolation made by Makemson.

Camera lucida drawings of the material from the parent and an aberrant culture were made (Plate IX). No material differences were found between the young growing mycelium of the parent and variant. The mycelium of the parent strain is about 2 μ wide, hyaline, quite delicate and of irregular septation, branching at a right angle or at an angle of 45 degrees. The cell contents are rather granular. The aërial mycelium of the variant is much wider (up to 4 μ in width), is very granular, and shows occasionally irregular inflations resembling chlamydospores. The mycelium, except for the uneven septation, resembles the conidiophores of the parent, but no conidia were ever seen on the variant form. The inflations mentioned have been described by Makemson (20) in normal cultures on corn meal agar. The parent form produced an abundance of conidia.

The striking difference between the variant and the parents, therefore, seems to be the lack of conidium production in the variant. The mycelium of the parent form is hyaline and the olive-brown appearance of the culture seems to be due to the conidia and the conidiophores. The variant form lacking these structures appears white or paler than the parent (Fig. 2 *c*). Light purple or pink color develops in the variant when grown on certain media (corn meal agar, prune juice agar, Czapek dextrose agar). The pink color appears most readily on alkaline media and with media rich in sugar. Table II give a comparison of the parent and variant forms on various media.

A study of the table reveals that both parent and variant showed different colors on the different media, but the variations were along parallel lines. The parent form varied along the olive-tawny shades, while the variant in the white-pink-lilac shades. The variant showed no tendency to develop patches distinct from the rest of the growth, while the parent form in various media continued to produce the "white islands." No spores were produced by the variant at any time on any medium.

EXPERIMENTS WITH SYNTHETIC MEDIA

In order to determine something of the influence of the nutrient salts and sugar on the color production, fruiting and

production of variants by the fungi under investigation, the following experiments were conducted.

Coons' synthetic solution (8) was devised with the aim of inducing sporulation. By increasing or decreasing the proportions of the salts above and below the optimum concentrations for this medium, a range of conditions was obtained. It was hoped that these experiments might further show whether it was the excess or lack of particular food material that induced the variations.

Coons' solution [1] is made of the following ingredients:

M/5 Maltose	5 cc.
M/5 Asparagin	1 cc.
M/5 Potassium acid phosphate . .	5 cc.
M/5 Magnesium sulphate (crystal)	1 cc.
Distilled water	88 cc.

By arranging the numbers of cubic centimeters of these nutrients (M/5) in a series according to the triangle system (25) and adding 2 per cent agar and enough water to make up 100 cc. of solution, fifteen solutions of varying proportions were obtained (Table III). The amount of magnesium sulphate was held constant in all the cultures. Maltose and potassium acid phosphate were used in greater and lesser amounts than the amount given in the regular formula. Asparagin was used in an increasing series. The solutions were designated by four-figure numbers in which the first digit stands for the number of cubic centimeters of M/5 maltose in 100 cc. of medium, the second for the number of cubic centimeters of M/5 potassium acid phosphate,

[1] This synthetic solution is made for laboratory use as follows:

Maltose	3.6 gms.
Asparagin	0.26 gms.
Potassium acid phosphate	1.36 gms.
Magnesium sulphate (crystal, $7H_2O$)	0.49 gms.
Distilled water to	1000 cc.

Steam on three successive days. *Do not autoclave.* The slight opalescence which develops when the solution is first made disappears rapidly. The pH of the solution is approximately 5.2.

Synthetic solution No. 2 has the same ingredients as the medium described above, but has five times as much asparagin.

the third for the number of cubic centimeters of M/5 asparagin and the fourth for the number of cubic centimeters of M/5 magnesium sulphate. For example, the number for the regular formula of Coons' medium is 5511, meaning 5 cc. M/5 maltose, 5 cc. M/5 potassium acid phosphate, 1 cc. M/5 asparagin and 1 cc. M/5 magnesium sulphate per 100 cc. of medium.

The ingredients were mixed in the correct proportions and the necessary amount of water containing melted agar was added to make 100 centimeters of medium. The amount of agar added in each case was sufficient to give a 2 per cent solution. The medium was tubed, steam-sterilized on three successive days and then slanted. It should be noted that in these experiments the reactions of the various combinations differed as did also the osmotic pressures. These experiments merely present a wide range of nutritional combinations.

Since in this experiment variations in color as affected by food supply were to be observed, as well as the behavior of the variant forms under different nutritional conditions, it was thought best to use solid media where the color could be better observed and compared. It was, therefore, impossible to determine the exact weight of mycelium produced. However, a similar series, but without agar, was prepared in 60 cubic centimeter Erlenmeyer flasks, each containing 20 cubic centimeters of the liquid media and *Colletotrichum lindemuthianum*, Exp. Sta. Strain II grown. This experiment will be described in detail further on.

Septoria apii, *Colletotrichum lindemuthianum*, Exp. Sta. Str. I and II, and *Cladosporium fulvum*, both parent and variant, were grown in duplicate on the triangle series of solid media as described above. The following is an account of the behavior of each fungus on these varying combinations of nutrients.

Septoria apii

There was no noticeable variation in color of this fungus grown on a set of media as described above. The fungus in all combinations of nutrients produced black mycelium with an abundance of pycnidia. There were differences in the amount of growth in the various media, as may be observed in the picture

of the set taken seventy days after inoculation (Plate X). The darkest color and heaviest mat developed in the cultures at the top of the triangle where sugar was present in the greatest proportion, 5421 and 6411 being the cultures making the best growth. It may also be seen that a small white tuft appeared on 3531 and 3621 of the base line of cultures. The decrease in the proportion of phosphorus seems to have influenced growth much more effectively than the decrease in the proportion of nitrogen in the form of asparagin, sugar and magnesium being constant. It should be noted that the potassium content varied also in these cultures, since potassium acid phosphate was the salt used.

Colletotrichum lindemuthianum, Exp. Sta. Strains I and II

Each strain was grown on a set of media as described above. The behavior of the two strains was similar. The tubes were inoculated with spores and the cultures were allowed to grow under natural conditions, side by side in the laboratory. The photographs (Pls. XI and XII) were taken seventy-five days after inoculation and give a good idea of the appearance. Acervuli with the characteristic salmon-pink spore exudate developed normally on all of them, independently of the black color, as, for example, in cultures with an excess of nitrogen, the black color developed very much later than the acervuli. The same thing had been noted previously with the synthetic medium No. 2. The greatest growth occurred in the cultures having the most sugar and potassium acid phosphate and there was a decrease in intensity of color as the amount of sugar decreased and phosphorus increased. Not a great number of white tufts developed. Several white patches were observed in culture 5511 (normal Coons' medium) of Strain I, but on being transferred to oat meal agar, corn meal agar and corn meal (flasks), they invariably became black.

The variations in the amount of growth may be better considered from the data of the liquid culture experiment. Twenty cubic centimeters of each medium in the set was placed in a sixty-centimeter Erlenmeyer flask and inoculated with 0.1

centimeter of a spore suspension of the fungus. The cultures in duplicate were grown at room temperature on clinostat for seventy-six days. At the end of this period careful notes were taken, the flasks were photographed (Pl. XIII), and their contents filtered on weighed filter paper. The mycelial mat and the filter paper were dried to a constant temperature and reweighed. Table IV records the weights of the duplicates in each medium, their average and descriptions of their growth and color.

The mycelium from the different flasks was examined microscopically. The black or brown pigment was found in the mycelium. The bodies referred to in the table above as black sclerotia were tiny tangles of mycelium. Sporulation was found correlated with the color. Brown or white mycelium showed no spores. Transfers from several flasks showing white mycelium to oat meal agar invariably became black. This seems to indicate that the variations in color were not fixed, but were due to nutritional factors which prohibited the formation of spores, especially towards the potassium-phosphorus side of the triangle.

Cladosporium fulvum

This organism was more responsive to the various nutrient combinations than any of the fungi under study. Both the brown and the white forms were grown on a set of media as described above and the results were very interesting, especially the variations in color. This fungus makes a slow and moderately abundant growth and it seems that there was enough food for its development in all the cultures, since there was very little variation in the amount of growth. The variations noted were (1) the color of the aërial part of the colony, (2) the substratum color and (3) the color of the submerged growth. The growth on media 5421 and 3441 seemed to be a little more abundant than on the others.

As regards the color diffusing through certain media, it had been noticed that the color on oat meal agar and corn meal agar was maroon and a similar pigment appeared on Media 4341 (Van Dyke red) and 3441 (faint Van Dyke red). These combinations are at the nitrogen corner of the triangle. In the rest

of the media the submerged growth was of a brownish-olive color tinged more or less with ochraceous-tawny especially towards the sugar side.

The color of the aërial part of the colony varied considerably according to the composition of the medium. In general it was brownish towards the sugar column, pinkish towards the nitrogen side and olive towards the potassium-phosphorus side. The intermediate cultures merged one into the other. White mycelium appeared in Media 5331, 5421, 4431, on the sugar-nitrogen side of the triangle. Table V gives the colors observed on the different media. The first line in each case is the surface color, the second the color of the submerged growth, the third the color variations in surface, the fourth the color diffused through the medium. The number after each color refers to the plate in Ridgway's *Color Standards*.

Cladosporium fulvum, variant form

The white strain of *Cladosporium fulvum* was also grown on a similar series of media. Table VI gives the colors observed on the different media. The growth was uniform in size of colony, 0.5 to 1 cm. in diameter, except on 3351 where the colonies were from 1 to 3 mm. in diameter. The white character of the variation was preserved throughout unchanged, except that a yellowish tinge developed towards the sugar side, purplish towards the nitrogen side and yellow-pink towards the potassium-phosphorus side. On account of the purple tinge the white was more striking upon the nitrogen side.

These tests with various combinations of nutrients threw very little light upon the problem under consideration. No particular combination seemed to influence the production of "white islands," although in several cultures with the various fungi the aberrant forms appeared. There was no regularity in their production. With *Cladosporium fulvum* a few "white islands" appeared in the cultures of the parent form, but in general these cultures showed the color typical of the growth on ordinary media — brown olive. The variant remained sharply differentiated in color production from the parent form.

EFFECT OF LIGHT ON VARIABILITY AND COLOR OF *CLADOSPORIUM FULVUM*

Microörganisms in general are negatively phototropic under strong light. Direct sunlight is detrimental to most bacteria although some fungi are resistant to it. Fungi grow well and fruit regularly in diffused daylight, which in many instances seems to be an essential factor for reproduction. Coons (8) found that with *Plenodomus fuscomaculans* light is a factor directly concerned with pycnidium production, the fungus refusing to fruit when kept in the dark, irrespective of nutrition, aëration, substratum or strain. Makemson (20) found that light had a retarding effect upon the growing germ tubes of *Cladosporium fulvum*, but that the ultimate length was the same. Vegetative growth and sporulation were also more profuse when the fungus was grown in the dark. Stevens (29), working with *Helminthosporium spp.* grown on Petri dishes, observed slightly less zonation and less aërial mycelium in the dark than in the light. Smith and Swingle (27) report that diffused daylight affected considerably the color of Fusaria, and Sherbakoff (20) found that diffused daylight intensifies the color of Fusaria, while intense light dulls it. They did not find light influencing other characters in Fusaria. Older literature reviewed by Coons (8) is just as contradictory. It seems that the different fungi vary in their responses to light of different intensities, some vegetating best in darkness and requiring the light stimulus to induce fruiting, others growing and fruiting best in diffused daylight or in darkness.

In order to determine whether light had any effect on color changes and variations of the fungi under study, four sets of media, as described in the preceding section, were inoculated with the white and brown strains of *Cladosporium fulvum*. A set of each strain was grown under a bell-jar completely darkened to exclude light. A second set of test tubes arranged and fastened on the walls of a battery jar, covered by a bell-jar, was exposed to strong diffused light and slowly rotated to overcome differences in intensity. Air was circulated through both bell-jars.

The jars were not disturbed until the end of the experiment, when the colors of the cultures in both sets on each medium were studied and pictures taken. A detailed description and comparison of the cultures on each medium in light and dark are not given, since the slight variations in color between the light and dark series did not seem consistent nor in any definite direction (see Pls. XIV-XVII).

In general light did not seem to affect markedly the color, growth of the colony or the number of "white islands," and it would seem that *Cladosporium fulvum*, both parent form and variant form, is indifferent to the light stimulus, and furthermore, light does not play any rôle in calling forth the formation of "white islands."

EFFECT OF REACTION ON VARIABILITY AND COLOR

The reaction of the substratum has recently been recognized as one of the most important environmental factors influencing the physiology of plants. It had formerly been thought that adjusting the reactions of the medium used, by some sort of titration, to a point above or below the turning point of an indicator, usually phenolphthalein, was a satisfactory method of securing an optimum reaction for microörganisms. However, with the discovery and perfection of methods for measuring the active acidity in media, investigations have shown that the hydrogen ion concentration should be classed with temperature, light and moisture as an important environmental factor. It was also found that, besides influencing growth in general, the reaction has an effect on color production, fruiting, zonation, and the like.

Sherbakoff (24) in 1915 found that acidity induces the production of red color in those Fusaria which make a grey-white growth on neutral media. Acidity also lowers the rate of growth and makes zonation prominent. Fungi are able to withstand a comparatively wide range of pH variation. Meacham (21) in 1918 found that *Lenzites sepiaria, Fomes roseus, Merulius lachrymans* and *Coniophora cerebella* would grow from pH 5-1.7 on the acid range. Webb (30) in 1919, studying the germination of spores of different fungi at different pH, found that the range of

spore germination with respect to the reaction of the medium is between pH 2–10 with maxima at pH 3–4 and 7. Johnson (16) in 1923 found that the reaction best suited for the growth of molds lies towards the acid range of the neutral point. Goss (14) in 1924 found that *Fusarium eumartii* is capable of growing under a wide variation in the hydrogen ion concentration on 2 per cent dextrose potato agar. At optimum temperature no appreciable difference was seen in the growth on media varying in pH from 4.5 to 8.5. Hopkins (15) in 1922 found that by adding 3 drops of 50 per cent lactic acid to 20 cc. agar, a strain to *Colletotrichum lindemuthianum* which produced few spores in neutral potato agar sporulated freely.

In order to determine the effect of reaction of medium upon the fungi under investigation, the following experiments were undertaken. Since the variations, "white islands," observed were characterized by a lack or modification of pigment and the suppression of sporulation, it was thought best to use two kinds of media; one favoring fruiting and the other favoring vegetative growth. Coons' medium was found by its originator actually to favor fruiting and the behavior of *Sphaeropsis malorum* in the present investigation also indicated the tendency of this medium to induce spore formation. Richards' medium E (23) has been frequently used by many investigators as a general synthetic medium with good results. The modification by Karrer and Webb (19) was used in order to avoid the precipitation of the phosphate in the alkaline members of the series. Liquid media were used in this experiment and the filter paper cone preparation dish method was adopted (8). This method consists in growing the fungus on a filter paper cone placed in a small preparation dish with 10 cc. of medium. Schleicher and Schull No. 595 (7 cm. in diameter) paper was used. The preparation dishes about 5 cm. in diameter and 3 cm. deep, were protected against contamination by placing them in large moist chambers or under bell-jars. Under such conditions the fungus grew on a solid substratum of pure cellulose kept at constant saturation. Both the white and the parent strains of *Cladosporium fulvum* and *Colletotrichum lindemuthianum*, Exp. Sta. Str.

II, were grown in duplicate series of ten different hydrogen ion concentrations varying from pH 2 to 8.4. Besides the paper cone cultures, the fungi were grown in an exactly similar series of media in test tubes with the proper indicator added to the medium. The purpose of this experiment was to determine as nearly as possible the shifting of the pH by the growth of the fungus.

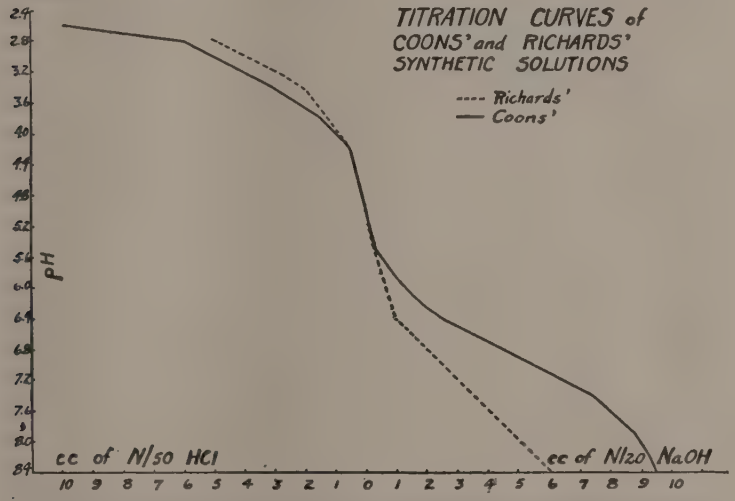

FIG. 3. Titration curves of Coons' and Richards' synthetic solutions

Previous to starting the experiment the titration curves of both media to be used were determined. Karrer and Webb (19) report a titration curve for Richards' medium E modified, but we were unable to duplicate their findings. The pH of Richards' solution is given by them as 4.6, but with the particular chemicals, apparatus, etc., with which the writers worked, it was found to be 5.0. This finding necessitated the retitration of the medium. Table VII gives the number of centimeters, the normality of acid and alkali, and the number of centimeters of water to be added to 25 cc. of double strength medium to make 50 cc. of medium of a desired pH and of the proper concentration in nutrients. It was found that by using weaker solutions of acid

and alkali than those recommended by Karrer and Webb (19), a smoother curve could be obtained. Figure 3 gives the titration curves. Table VIII shows the change in uninoculated media after one and a half months. In general the Coons' medium held constant, while the Richards' medium in the alkaline range became weakly acid.

The preparation dishes with the filter paper cones in place were sterilized in the dry air sterilizer while the medium, the water and the acid or alkali, were autoclaved separately. The proper amounts were mixed and pipetted into the tubes and the preparation dishes by means of sterile pipettes. A suspension of spores was used as inoculum in the case of the parent strains of *Colletotrichum lindemuthianum* and *Cladosporium fulvum*, but in the case of the variants of both fungi a bit of mycelium was used.

Cladosporium fulvum

The cultures were grown in the dark. Twelve days and again in one and a half months after inoculation, the color, the relative amounts of growth and in the case of the test tube cultures, the pH, were noted. Tables IX and X summarize these observations for test tube cultures, and Table XI summarizes the results obtained with filter paper cones.

With the Coons' medium the parent form grew well on a range of pH 2.8 to pH 7.6, producing in the submerged portions the dark olive-green color typical of the submerged growth of this organism on ordinary media. The aërial growth of this was of a light yellow-olive color. A purplish color (vinaceous-lilac, daphne-red) was produced towards the alkaline side of the series and this color is characteristically produced in old cultures on oat, corn meal and prune juice agar. The same purplish color was observed in the variant strain also in the alkaline cultures. "White islands" appeared in both acid and alkaline cultures with Coons' medium.

There was a greater variation in the color of the aërial growth on the Richards' series, but there was no characteristic distribution, the same color being found on both the acid and the alkaline side. No purple tints were found on either the parent or the

variant with this medium. On the contrary the buff color characteristic of the variant when grown in acid media was found throughout the series on this medium. It is not sure that the alkaline tubes of Richards' medium remained in this condition under the action of the air, since the solution is poorly buffered. In general, as has been observed by many investigators, fungi given abundant carbohydrate tend to shift the reaction of an alkaline substratum towards the acid side and this was the case in the experiments just described. After one and a half months final notes were taken. It will be seen that in the case of both forms the tolerance of acid and alkaline conditions is practically the same; the effects of the parent and variant strain upon the medium, taking into account the relative growth, are alike. The distinctions in color are maintained; and what is perhaps the most significant, in spite of the great variety of growth conditions presented, there is a complete absence of sporulation in the variant as contrasted with heavy spore production in the parent forms in some cultures. The differences of the two forms manifested in the previous work seemed to be largely maintained.

Colletotrichum lindemuthianum

Similar observations were made on the parent strain and the variant of *Colletotrichum lindemuthianum*. These results are tabulated in Tables XII and XIII, for the Coons' and the Richards' media respectively. It will be noted that the parent form produced spores throughout the range in which it grew, whereas the variant form produced no spores at all. The growth range was approximately the same, with slight variations in amounts of growth produced. On filter paper cones saturated with Coons' medium the fungus seemed to revert to the parent form, so far as vigor of growth and color of mycelium are concerned (Table XIV). No spores were found, however. These results seem rather significant in view of the uniform white growth in other tests of the fungus on various media and under other conditions, and seem attributable to the more favorable conditions for growth afforded by the use of the filter paper cones

which doubtless favor aëration, removal of toxic byproducts and other growth-controlling factors.

EFFECT OF TEMPERATURE AND REACTION ON COLOR AND GROWTH

Cladosporium fulvum

In connection with another experiment, carried on in Berkeley, California, both parent and variant strains of *Cladosporium fulvum* were grown on plated Shive's best dextrose agar [2] (26) varying in pH from 4 to 8 and kept at 30–32° C., 20° C. and 10° C. The medium was prepared in lots of one liter, brought to the desired hydrogen ion concentration by the addition of acid or alkali at rates determined by Sideris (26), tubed at the rate of exactly 10 cc. per tube and steamed for one hour on three consecutive days. Duplicate plates were poured, allowed to cool and inoculated in the center. They were wrapped in paper and kept at a constant temperature, as mentioned above. At intervals the plates were examined for color and rate of growth.

Table XV gives the growth of the fungus (diameter of colonies in millimeters) and color of the aërial and submerged part, three, eight and fifty-four days after inoculation at the three temperatures used.

Makemson (20) found that the temperature limits for best growth of this fungus were rather narrow, being between 20° and 25° C. In this experiment no growth occurred at 10° C. and only slight growth was noticeable after fifty-four days. Temperature, therefore, did not enter as a factor in this experiment. The variant was found to be a more active grower than the parent strain, as indicated by the colony measurements, and both parent and variant seemed to grow rather uniformly within the hydrogen ion concentration limits of the experiments.

[2] The composition of this medium is as follows:

$MgSO_4$	2.12 gms.
$Ca(NO_3)$ 2 per cent	.71 gms.
KH_2PO_4	1.36 gms.
$FeNO_3$ 5 per cent	1 drop
Dextrose	20 gms.
Water	1000 cc.

As regards the color, this experiment confirms the results of the previous experiments on the effect of the reaction on the medium. No striking variations were seen even between the extreme ends of the series, in either the variant or the parent, except perhaps the development of the purple color in the variant towards the alkaline end, as had also been observed in previous experiments. A repetition of this experiment gave similar results.

PATHOGENICITY

The majority of the variations induced through unfavorable environmental conditions have been among the saprophytic fungi and bacteria. Of the spontaneous variations or mutations reported in pathogenic fungi a few do not show reduced pathogenic action, though in others the reverse is true and in still others data are lacking.

Edgerton (13) found that the Glomerella mutant reported by him grew very slowly on apple. The Brachysporium mutant reported by Bonar (3) was not pathogenic on white clover and came from a parent of reduced virulence due to artificial cultivation for many generations. Bennett, who, according to Coons (1), investigated white forms developed from a culture of *Phoma apiicola*, found that they are also attenuated forms. On the other hand Crabill's Coniothyrium variant (10) was pathogenic, as also was the Glomerella variant reported by Dastur (11) and the Botrytis reported by Brierley (4). Definite information is lacking in the cases reported by Burger (6) and Stevens (29).

Cladosporium fulvum

Four potted plants of the Stone variety of tomato were used in these experiments. Two of them were inoculated with the parent strain, and the other two with the variant strain of *Cladosporium fulvum*, the inoculations being made in separate rooms of the laboratory under the usual aseptic conditions. A drop of sterile water placed on the lower or upper surface of a leaflet, was inoculated with a little mycelium from a young vigorous culture and covered with a fleck of sterile cotton.

Many leaflets were inoculated in this manner. Each plant was then put under a large glass bell-jar for five days and kept exposed to light near a north window of the laboratory at 20–25° C. After this period the plants were brought into the greenhouse. The experiment was repeated with four potted plants of the Beauty tomato variety.

The results of the inoculations were as follows: Both the parent and the variant produced spots at every point of inoculation, more than thirty spots being counted in each case. No spots were seen at other than the inoculation points and although these plants, together with many control plants, were kept in the same greenhouse there was no natural infection. The spots produced by the parent strain were all characteristic of the disease as described by Makemson (20). There was an olivaceous growth on the under side of the leaf with an abundance of spores characteristic of the fungus. The upper surface of the leaf in the infected area turned yellow and became dry. The spot spread irregularly from the point of inoculation.

The spots produced by the variant strain were elliptical and well defined, rather dry with yellowish flakes on the surface. There was no mycelial growth on the under side of the leaf. Free-hand sections through the infected portion of the leaf showed the presence of mycelium throughout the leaf tissue. Five plates were poured from leaves infected by the variant and Czapek dextrose agar was used for a medium. In every case the white form of Cladosporium grew on these plates. Similarly infected leaves were placed in moist chambers. The white variant grew from the margin of every spot. Plates from tissue infected by the parent strain showed an abundance of growth of the typical *Cladosporium fulvum*.

Plate XVIII shows leaflets of tomato artificially infected with both forms of Cladosporium. Isolations were made from the part of the leaf infected both by the parent and the variant strains and reinoculations were made on potted Beauty tomato plants in bloom. The methods used were those described above, except that inoculations were made on both the upper and lower sides of the leaflet. No spots were produced from upper-surface

inoculations. Typical spots similar to those described above developed on all (fifteen) of the lower surface inoculations. Inoculations on the calyx and the stem of flowers produced characteristic spots. Small tufts of white mycelium were found at the margin of the spots produced by the variant. Such tufts were examined under the microscope for spores. There were no typical spores except a few spore-like bodies believed to be chlamydospores. The mycelium was thin, densely branched, and purple.

The parent form of *Cladosporium fulvum*, although it had been under artificial conditions for over four years, produced the typical leaf mold disease.

Dastur (11) found that passing the variant form of Glomerella through its host restored its lost spore-producing power, but this was not evidently the case with *Cladosporium fulvum*, since it did not produce spores even after passing through its host a second time. From the experiments described above, it becomes evident that both the parent and variant are parasitic on the tomato plant, but passage through the host does not immediately restore the color or fruiting capacity of the variant.

Colletotrichum lindemuthianum

Golden Wax bean plants were inoculated with the parent and variant form of *Colletotrichum lindemuthianum*, Exp. Sta. Strain II; spores or bits of mycelium were used in the same manner as in the tomato plant inoculations. Inoculations were made on both the upper and the lower surface of the leaves. No spots were formed on any of the upper-surface inoculations or on the control leaves. The results were not as definite as in the case of Cladosporium. The leaflets inoculated with the white form of Colletotrichum turned yellow, but no definite spots developed except in one case where typical anthracnose spots were observed. No spores were found. The leaflets inoculated on the under surface with the black form of Colletotrichum showed typical spots of anthracnose with an abundance of spores.

SUBSEQUENT HISTORY OF THE CULTURES

In August, 1923, culture work with these forms was discontinued at the laboratory and the cultures were carried along by frequent transfers as stock cultures. The results of these transfers are as follows:

Examinations made in 1924 and early in 1925 showed that the marked difference between the parent strain and the variant had largely disappeared. The variant had practically the color of the parent form and on some media produced more nearly the typical color of normal *Cladosporium fulvum* than the parent form. The parent form had ceased to sporulate in our cultures and no spores have been found in cultures of the variant strain. An interesting development was the production of "white islands" in the cultures of the variant form. On subsequent transfers the variant form continued to develop the color normal for *Cladosporium fulvum*.

It is our opinion that these forms are not as distinctly different as when the first isolations were made.

It would seem that these forms, though markedly different when isolated, if supplied with proper conditions, return to the parent form, or at least to what approaches it fairly closely.

It will be noted from the experiments that *Septoria apii*, *Sphaeropsis malorum*, *Colletotrichum lindemuthianum* and finally *Cladosporium fulvum* have all given off sterile mycelia which have been of different color from the parent strain. All these have eventually reverted to the normal or nearly the normal form. The case is not completely clear since sporulation has not been obtained with the several variants, but we would question strongly any "specific" difference between the variant and parent forms under observation.

DISCUSSION

From the experiments here outlined the following statements can be made:

1. In single-spore cultures of a number of fungi, variants occurred repeatedly. Those with which we have been concerned

have been the white forms — the sterile patches cropping up here and there in cultures. The sterile patches we have called "white islands."

2. Various modifications of the environmental factors have been tried and these have exercised profound effects on the colors and fruiting habits of the various fungi, especially *Cladosporium fulvum*. These modifications have not, however, been found to be the essential factors inducing the type of variation under consideration. The white forms, on the contrary, have come at unprognosticated and irregular intervals in the various strains under observation.

3. When once isolated, some of these variants have been strikingly constant in spite of culture under a wide range of conditions, such as variations in type of substratum, variation in percentage composition of medium and even life on the host as an active parasite.

4. On the other hand some isolated variants apparently revert at once to the normal form. Others, as in the case of *Colletotrichum lindemuthianum*, Strain II, after long sojourn in culture suddenly revert to the parent or at least to the color of the parent form. The most constant of the forms under investigation, the atypical form of *Cladosporium fulvum*, remained for two years entirely distinct from the parent. The striking thing was its change back to normal color by subsequent culture on a different medium (potato dextrose agar) from the ones tried in the experiments. As yet sporulation has not occurred. The parent culture in the meantime has ceased to sporulate. An interesting development is the production anew of "white islands" in the reverted form.

The nature of variation in plants and animals is a subject which in the last hundred years has received great attention and today is occupying by far the greatest share of attention at the hands of biologists. That mutations do occur in apparently pure line cultures of plants and animals is attested in a great variety of contributions covering the whole range of plant and animal life.

The significance to be attached to the various contributions depends in large measure upon the definition of the problem

and upon the criteria of purity established in the cultures. De Vries in his monumental work on *The Mutation Theory* does not make any discrimination between the terms 'saltation,' 'mutation' or 'sport' and he uses them more or less interchangeably. The Darwinian term 'single variation' is used to designate the same thing. The essential element in the De Vriesian concept was the suddenness of the change, its constancy, and its difference from the ordinary fluctuating variation brought about by environmental influence.

But the work of De Vries preceded the vast amount of cytological and genetic work and the modern concept of mutation has been of necessity molded in its interpretation by the later contributions. Today by common agreement, mutation is taken to mean a change in the *genetic constitution*, or, as Brierley (5) describes it, "a genotypic change in a pure line." In his thoughtful essay, *Some Concepts in Mycology*, Brierley has established the following criteria of a true mutation:

> There are certain evident minimal requirements for any studies to this end which may perhaps be expressed as follows:
>
> 1. In order to ensure specific purity the organism must be a single individual of a tested pedigree pure line.
> 2. The whole life-history of the organism, together with the range of its plasticity both morphological and physiological, must be accurately known in the minutest detail.
> 3. No organism in which sexuality exists, or it is conceivable that it may exist, must be used unless its gametic constitution and genetic behavior under all conditions of the experiment be known.
> 4. Possible contamination by filterable gonidia must be eliminated.
> 5. Adequate control experiments must be maintained (a little matter but one absolutely vital, which has escaped the attention of many students of the lower organisms).
>
> Unless these five conditions are rigidly maintained in the focus of one's attention, and exactly complied with, the results obtained in experimental studies on the educability of microorganisms can have but little value.

The variations of fungi and bacteria when grown in apparently pure line cultures recorded in the literature are numerous. These variations range from those quickly reverting forms to apparently stable forms which various authors have denoted as mutants. Other names have been employed — saltations, sports, and variants being used by various authors and

with varying connotations. The literature of variation in fungi has been carefully summarized by Brierley (4) and Stevens (29) and the extensive bacteriological contributions have been exhaustively summarized by Dobell (12), Jordan (17) and Jollos (18). This mass of literature needs no extensive review. The various authors have attempted to fit the terminology and criteria employed to those in vogue with higher plants. It is a common property of much of this work that several assumptions have been made, namely, that the cultures used have been pure lines representing homozygous entities and that with the bacteria, at least, the material dealt with has concerned itself with organisms in which sexuality and cell fusions need not be considered.

It is to be noted that Brierley (4) in his work with Botrytis has in a remarkable manner departed from those fixed assumptions and, in the absence of cytological evidence, he declines to consider as a true mutant the white sclerotial form developed in his culture of Botrytis, but rather explains the development of the aberrant form as resulting from contamination from some preceding anastamozing or fusion of cells. Similarly, Stevens (29) employs the term saltation to cover variations in an organism where cytological conditions and sexuality are unknown.

Variations in fungi have been induced through exposure of cultures to unfavorable environmental conditions, such as high temperature, toxic substances, unbalanced nutrients and the like, but few if any of these contributions meet the standards erected by Brierley. In part the earlier work has dealt with selections of those individuals in the culture which were resistant to extremes and, in the absence of exact knowledge of the nature of the parent culture, may be regarded as the separation of the culture with its pure lines.

New developments in science bring about modifications of old notions. Brierley (5) has insisted that possible contamination by "filterable gonidia" must be eliminated. The recent work on the "bacteriophage," or transmissible lytic principle, makes very clear the importance of close scrutiny of the bacteriological material in order to be sure that "pure" cultures are ultra-pure. No intimation exists in the literature that we are to suspect

fungous cultures of being similarly contaminated, but such a possibility must not be overlooked.

One can but agree with De Vries that nothing is more variable than the meaning of the word variability and perhaps the word 'mutation' may be substituted for 'variability.'

The confusion of ideas surrounding the term has lead several writers to avoid it altogether. Morishima (22), in 1921 reporting several cases of adaptive changes in bacteria, *B. typhosus* in particular, thinks that the term mutation should be used only with higher plants and should not be introduced into bacteriology "for the bacteriologist, who studies his species not only from the morphological point of view, but also with regard to biochemical and immunological reactions, and who observes not a few generations only, but hundreds and thousands of generations, would almost surely have to modify the conception of the term in such a manner as to cause confusion to the botanist. It, therefore, seems advisable to leave the term mutation to the botanist and, for the present at least, to speak of atypical varieties of bacteria or simply variants."

Stevens (29) uses the term saltation with a new meaning to cover variations in non-sexual generations of fungi. Chaudhuri (7) follows his example for the same reason.

By nearly all the criteria ordinarily employed in judging "mutants," it might seem that in this work we are dealing with definite mutant forms. Had the work stopped after a few transfers, such a status might have been claimed. Nothing striking in the way of a new species had been produced, the condition inducing the production was unknown, but, nevertheless, the forms were atypical, were easily recognizable from the parent form, and were constant enough in culture to satisfy ordinary standards. In addition marked differences in pathogenicity to the host were obtained.

But the reversion of these fungi to the parent form or what closely resembles it, is in our opinion the striking thing in the behavior of these strains. Some reverted at once to the normal, others after many transfers, and *Cladosporium fulvum* after years of cultures on media.

It is the opinion of the writers that the variants dealt with in this paper represent rather semi-permanent variations which are different from the parent form somatically rather than genetically. These are the "dauer-modifikations" of Jollos (18), which perhaps by drying of the mycelium and lack of nutritional connection with the substratum, have become changed from the normal. With respect to certain attributes they may be looked upon as attenuated forms. Instead of being new species they are rather to be looked upon as cultures lacking certain physiological powers. They resemble in some respects the *Abkulturs* of Fusaria and the so-called attenuated cultures of bacteria and fungi. They may have arisen from cells whose protoplasm has been poisoned, or perhaps affected by some unknown biological factor, and which are tardy in recuperation until supplied with the necessary conditions. This study illustrates that the criteria established for proof of the educability of fungi are necessary and that what may seem plausible evidence of the establishment of new forms in pure cultures needs careful and protracted investigation.

SUMMARY

White variants were isolated from known single-spore cultures of *Septoria apii*, *Sphaeropsis malorum*, *Colletotrichum lindemuthianum* and *Cladosporium fulvum*. The variants of *Septoria apii* and *Sphaeropsis malorum* reverted at once to the parent form. From the other two fungi strains were obtained which remained constant on laboratory media for over two years. These forms were lacking in the color characteristic of the parent forms and did not sporulate.

Nutrients, light and dark, reaction of substratum were not found to be the controlling factors leading to production of the variants.

The variant forms remained fixed under a wide range of environmental conditions.

The variant forms were pathogenic to their respective hosts, but the disease produced by the *Cladosporium fulvum* variant was not like the typical disease produced by *Cladosporium fulvum*.

All the forms eventually have regained the color of the parent form, but not the capacity for spore production.

These variants are looked upon as modified forms whose behavior is analogous to that of *Abkulturs* of Fusarium and the so-called attenuated cultures of fungi and bacteria.

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TABLE I

THE APPEARANCE OF ACHROMATIC VARIATIONS IN FOUR SINGLE-SPORE STRAINS OF *Cladosporium fulvum* Cke., OTHERWISE IDENTICAL, ON A SERIES OF STANDARD MEDIA

Positive cases marked: ++ ; negative: −−.

MEDIA	Strain I	Strain II	Strain III	Strain IV
Corn meal agar				++
Corn meal flask	−−	−−	++	++
Rice flask	−−	−−	−−	−−
Prune juice agar	−−	−−	−−	−−
Oat meal agar	++	++	++	−−
Synthetic agar No. 2	++	−−	++	−−
Coons' agar				++
Richards' liquid	−−			
Carrot plug Potato plug	−−	−−	−−	−−
Nutrient agar				++

TABLE II

GROWTH AND PIGMENTATION OF PARENT AND VARIANT STRAINS OF *CLADOSPORIUM FULVUM* ON SEVERAL MEDIA

	PARENT					VARIANT			
	Growth	Spore product	Variations	Color		Growth	Spore product	Color	
				Surface	Through medium			Surface	Through medium
Corn meal agar	++	+++	−	Buckthorn-brown	Dark livid purple	++	−−	White	Astor purple
Corn meal flask	+++	+++	+	Dresden brown		++	−−	White	
Oat meal agar	+++	+++	+	Metal bronze	Raisin-black	++	−−	White	Burnt-lake
Prune juice agar	+++	+++	+	Argus brown	Raisin-black	++	−−	Pale pinkish-buff	Dusky dull violet
Nutrient agar	+++	+++	−	Deep grayish-olive	Chaetura black	++	−−	White	Vinaceous
Rice flask	+++	+++	+	Brussels brown	Dusky olive-green	+	−−	White-shiny	
Carrot plug	+++	+++	−	Saccardo-umber	Dark				
Coons' synthetic agar	+++	+++	−	Brussels brown	−	+++	−−	White	
Synthetic agar 2	+++	+++	+	Benzo-brown	Dusky purplish-grey	++	−−	White	Citrine
Czapeck synthetic agar	+++	+++	+	Deep olive-grey	Vinaceous-grey	++	−−	White	Dark heliotrope-grey
Shive's best synthetic agar	+++	+++	+	Buffy-citrine	Mumy-brown	++	−−	White	Hessian brown
Lima bean agar	+++	+++	+	Mouse-grey	Olivaceous-black	++	−−	White	Hessian brown
Tomato stems	+++	+++	−	Brownish-olive	Purple hyphae	++	−−	White	

TABLE III

ARRANGEMENT OF CULTURES ACCORDING TO TRIANGLE SYSTEM

Figures represent quantities of M/5 chemicals

cc. M/5 Maltose per 100 cc. 1st figure

				7311 oo						
			6321 oo			6411 oo				
		5331 oo			5421 oo			5511 oo		
	4341 oo			4431 oo			4521 oo			4611 oo
3351 oo			3441 oo			3531 oo			3621 oo			3711 oo

cc. M/5 Asparagin per 100 cc. 3d figure

cc. M/5 Potassium acid phosphate per 100 cc. 2d figure

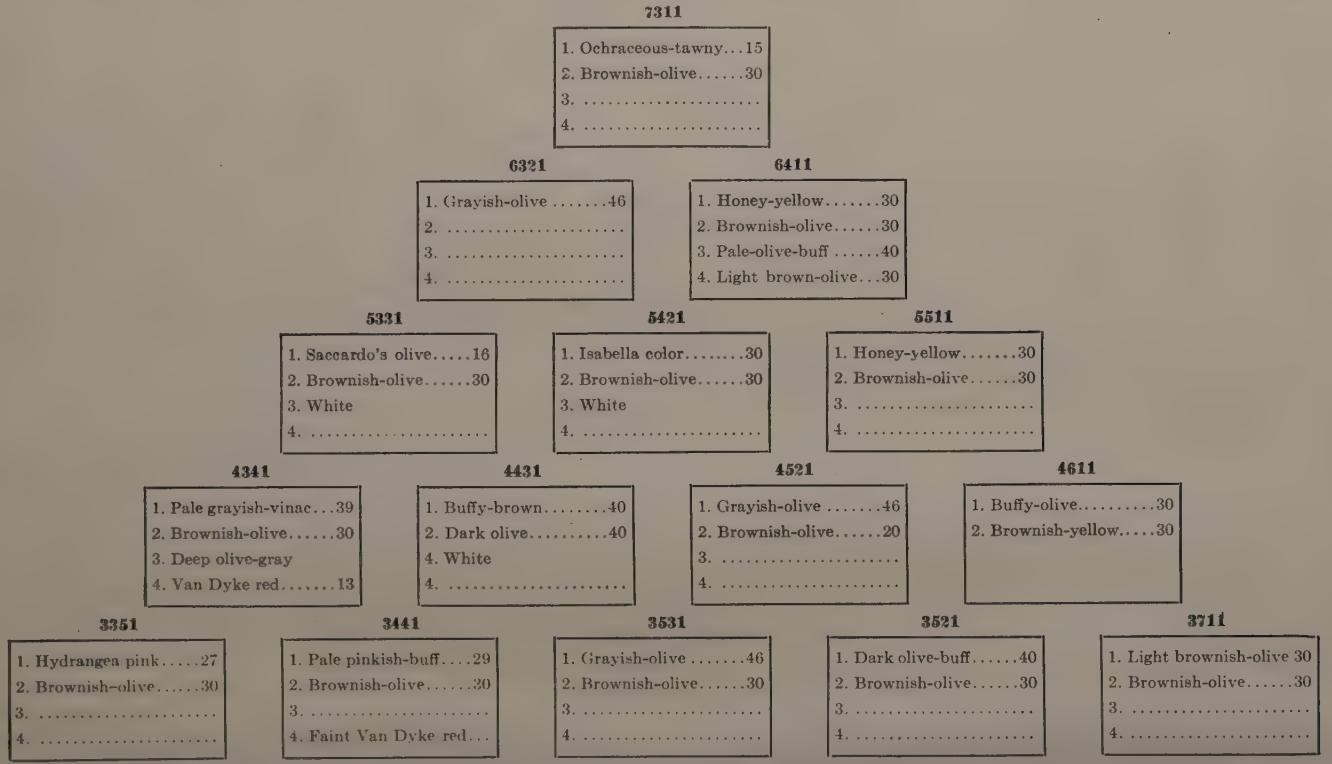
TABLE IV

WEIGHTS OF *COLLETOTRICHUM LINDEMUTHIANUM* WHEN GROWN ON LIQUID COONS' MEDIUM VARIED ACCORDING TO THE TRIANGULAR SYSTEM, TOGETHER WITH A DESCRIPTION OF THE CULTURES

MEDIUM	1st Ser. mgs.	2d Ser. mgs.	Average mgs.	DESCRIPTION
7311	17.30	23.00	21.10	Abundant growth, submerged mycelium dirty white, a ring of Natal brown adhering to the glass on the surface of the medium
6321	9.9	9.0	9.4	Wide blackish-brown ring around the edge, with black small sclerotium-like formations scattered through it. Submerged mycelium white
6411	15.5	25.8	20.2	No ring, no sclerotia, white mycelium, Royal-brown patches on the surface
5331	.8	13.5	6.3	Abundant white mycelium. Concentrically arranged sclerotia on the surface. Narrow, black ring discontinuous
5421	4.7	7.1	5.9	Mycelium brownish. Concentrically arranged sclerotia on surface. Discontinuous black ring
5511	10.1	10.0	10.0	Mycelium pale brown. Ring indefinite
4341	4.7	12.8	8.7	Mycelium pure white. Discontinuous, loose black ring
4431	4.0	6.3	5.1	Mycelium nearly white. Wide, compact, blackish-brown, discontinuous ring
4521	5.9	4.2	5.0	Mycelium dirty white. Black, compact 1 cm. in diameter patches on the surface
4611	2.7	6.3	4.5	Mycelium brownish. Indefinite ring brown with sclerotia
3351	2.0	11.0	6.5	White mycelium. Thin ring with sclerotia
3441	6.4	2.1	4.2	Mycelium nearly white. Thin ring with sclerotia
3531	2.0	4.8	3.3	Mycelium brown. Thick brownish ring
3621	3.0	4.0	3.5	Mycelium brown, ring indefinite, sclerotia
3711	ooo	2.9	2.9	Mycelium brown, ring indefinite, sclerotia

TABLE V. Color Variation of *Cladosporium fulvum* (Parent Strain) grown on Coons' Medium Varied in Composition according to the Triangular System

Sugar (Maltose) 1st Figure



Asparagin, 3rd figure

Phosphorus-potassium, 2d figure

1. Surface color
2. Color of submerged growth
3. Color variation on surface
4. Color diffused through the medium

TABLE VI. Color Variation of *Cladosporium fulvum* (Variant Form) grown on Coons' Medium Varied in Composition according to the Triangular System

Sugar (Maltose) 1st Figure

7311

1. Cream color.....16
2. Buckthorn-brown...15 (on top of slant)
3. .....
4. Brownish-olive.....30

6321

1. Buffy-brown.....40
2. White
3. Fawn.....40
4. .....

6411

1. Tillent buff.....40
2. .....
3. .....
4. Dusky olive-gray....41

5331

1. White
2. Cameo pink.....26
3. Deep vinaceous....27
4. .....

5421

1. Seashell-pink.....14
2. White
3. Warm sepia.....29
4. Mars yellow.....3

5511

1. White
2. Pale olive-buff.....40
3. Brownish-olive.....30
4. .....

4341

1. White
2. Pale lilac 37
3. Mars violet.....38
4. Ochre red.....27

4431

1. White
2. .....
3. Vernonia purple.....36
4. .....

4521

1. White
2. .....
3. Cinnamon-brown
4. .....

4611

1. White
2. .....
3. Light brown-olive...30
4. Brownish-olive.....30

3351

1. White
2. Dark vinaceous-purple 36
3. Dark olive 40
4. .....

3441

1. White
2. Pale lilac.....37
3. Vinaceous-purple....38
4. .....

3531

1. White
2. Pale lilac.....37
3. Mars violet
4. .....

3521

1. White
2. .....
3. Corinthian purple...38
4. .....

3711

1. White
2. .....
3. Brownish-olive.....30
4. Brown-olive.....30

Asparagin, 3rd figure

1. Surface color
2. Exception or tinge
3. Color of colony as seen through the slant
4. Color of the submerged growth

Phosphorus-potassium, 2d figure

TABLE VII

Titration of Richards' and Coons' Media

Centimeters of acid or alkali and water to be added to 25 cc. of double strength medium to obtain a series of media of varying pH and proper concentration of nutrients

pH	cc. HCl	Normality	cc. NaOH	Normality	cc. H_2O	Volume
Richards'						
2.0	11	N/20	**	**	14	50
2.8	7	N/50	**	**	18	50
3.7	1.5	"	**	**	23.5	50
4.8	.25	"	**	**	24.75	50
5.0	**	**	**	**	25	50
5.8	**	**	.5	N/30	24.5	50
6.4	**	**	1.0	"	24	50
7.0	**	**	2.5	"	22.5	50
7.6	**	**	4.0	"	21	50
8.4	**	**	6.0	"	19	50
Coons'						
2.4	20	N/50	**	**	5	50
2.8	8	"	**	**	17	50
3.2	4	"	**	**	21	50
3.8	1.5	"	**	**	23.5	50
4.2	.5	"	**	**	24.5	50
5.0	**	**	**	**	25	50
5.6	**	**	.5	N/20	24.5	50
6.4	**	**	2.5	"	22.5	50
7.0	**	**	5.5	"	19.5	50
7.6	**	**	7.5	"	17.5	50
8.4	**	**	9.5	"	15.5	50

TABLE VIII

CHANGES IN UNINOCULATED MEDIA AFTER ONE AND ONE-HALF MONTHS

Coons' Medium + Indicator			Richards' Medium + Indicator		
	Start	Final	Indicator	Start	Final	Indicator
T.B.	2.4*	2.4	T.B.	2	2	
T.B.	2.4	2.4	T.B.	2		
T.B.	2.8	2.8	T.B.	2.8		
T.B.	2.8			2.8		
BPB	3.2	3.2	BPB	3.7		
BPB	3.2	3.2	BPB	3.7	3.7	
BPB	3.8	3.8		4.8	4.8	
BPB	3.8			4.8	4.8	
MR	4.2			5.0	5.0	
MR	4.2			5.0	5.0	
	5.0	5.0	MR	5.8		
	5.0			5.8	5.6	BCP
	5.6	Faded	MR	6.4		
	5.6	Faded	MR	6.4		
	6.4	6.4	BTB	7.0	6.4	BTB
	6.4			7.0	7.0	BTB
	7.0			7.6		
	7.0			7.6	Approx. 6.6	more acid than PR range
				8.4		
				8.4	6.6	BTB
	8.4	8.4	T.B.			
	8.4					

* Initials refer to standard Clark and Lubs indicators

TABLE IX

COMPARISON OF THE TWO STRAINS OF *CLADOSPORIUM FULVUM* IN COONS' MEDIUM OF VARYING HYDROGEN ION CONCENTRATION: CULTURES IN TEST TUBES

PARENT FORM					VARIANT FORM				
Initial pH	Growth after 1½ mos.	Color after 1½ mos.	pH after 12 da.	pH after 1½ mos.	Initial pH	Growth after 1½ mos.	Color after 1½ mos.	pH after 12 da.	pH after 1½ mos.
2.4	±	(Merely starting)	2.4	2.4	2.4	±	(Merely starting)	2.4	2.4
	±	"	2.4	2.4		±	"		2.4
2.8	+	Olive-grey	2.8	2.6	2.8	+	Bluish gray-green		3.0
	+	" "		2.8		+	" "		3.4
3.2	++	Warm buff		3.2	3.2	++	Light buff	3.2	3.4
	++	" "		3.2		++	" "		3.4
3.8	+++	Tawny	3.6	4.0	3.8	++	Light buff	3.6	4
	+++	"		4.0		++	" "		4.2
4.2	++++	Buckthorn-brown *	4.2	4.6	4.2	+++	Dark (submerged)	4.2	4.6
	++++	Buckthorn-brown		4.4		+++	" "		4.6
5.0	++++	Buckthorn-brown †	5.0	5.2	5.0	++	Light buff	5.0	4.8
	++++	Buckthorn-brown †		5.2		++	" "		4.8
5.6	+++	Buckthorn-brown	5.6	5.6	5.6	+++	Light buff	5.6	5.4
	+++	" "		5.6		+++	" "		5.4
6.4	++	Buckthorn-brown ‡	6.4	6.2	6.4	++	Light buff	6.4	6.2
	++	" "		6.4		++	" "		6.2
7.0					7.0				
7.6	++	Buckthorn-brown	7.2	7.2	7.6	++	Light tan	7.2	7.2
		" "		7.2					
8.4	+	Russet-vinaceous		7.4	8.4	++	Pinkish-white	8.4	6.8
						++	" "		7.6

* 1 "White island" † 4 "White islands" ‡ 2 "White islands"

TABLE X

COMPARISON OF THE TWO STRAINS OF *CLADOSPORIUM FULVUM* ON RICHARDS' MEDIUM OF VARYING HYDROGEN ION CONCENTRATION: CULTURES IN TEST TUBES

PARENT FORM					VARIANT FORM				
Initial pH	Growth after 1½ mos.	Color	pH after 12 days	pH after 1½ mos.	Initial pH	Growth after 1½ mos.	Color	pH after 12 days	pH after 1½ mos.
2.0	0		—	2.0	2.0	0		2.0	
	0			2.0		0		2.0	
2.8	+	Light olive-gray	2.8	2.5	2.8	+	(Submerged)	2.8	2.5
	+	"	2.8	2.5		+	Light gull-gray		2.5
3.7	+	Gray	3.5	3.2	3.7				
	+	Light olive-gray	3.5	2.4		+	Pale olive-gray	3.5	2.8
4.8	++	Tea-green	4.6		4.8	++	Ivory-yellow	4.6	2.8
	++	"	4.6	2.7		++	Light grayish-olive		2.8
5.0	+	Mineral-gray	4.8	2.6	5.0	++	Olive-buff	4.8	2.6
	+	" "	4.8			++	Vinaceous-buff		2.4
5.8	++	Tea-Green	5.4	2.6	5.8	+++	Ochraceous-tawny	5.4	2.8
	++	" "	5.4	2.6		+++	" "	5.4	2.4
6.4	++	Olive-lake		3.6	6.4	++++	Gray		2.2
	++	" "		2.4					2.4
7.0	++	Light olive-gray	6.6	3.2	7.0	++	Pale olive-buff	6.6	2.4
	++	Olive-gray	6.6	2.6		++	" "	6.6	2.5
7.6	++	Olive-gray	7.0	2.6	7.6	+++	Pinkish-buff	7.0	2.4
	++	Olive-gray	7.0	2.6	7	+++	" "		2.4
8.4	++	Olive-gray		4.1	8.4	+	Pinkish-buff		4.4
	++	Olive		2.5		+	" "		3.5

TABLE XI

COMPARISON OF THE TWO STRAINS OF *CLADOSPORIUM FULVUM* ON COONS' AND RICHARDS' MEDIA OF VARYING HYDROGEN ION CONCENTRATION: CULTURES IN PREPARATION DISHES WITH FILTER PAPER CONES

RICHARDS' MEDIUM

PARENT FORM				VARIANT FORM			
pH	Growth	Color	Spores	pH	Growth	Color	Spores
2.	– –			2.			–
2.8	Contam. +	Dark gray-green	±	2.8	+ +	Greenish "	– –
3.7	Contam. ++	Dark gray-green	+	3.7	Contam. +	Tan	–
4.8	+ ++	Blue-green Greenish	+	4.8	+ +	Tan "	– –
5.0	+ +	Olive-green " "		5.0	Contam. "		
5.8	+ +	Olive-green Greenish-black	+	5.8	+ +	Pale vinaceous-lilac Growth at edge greenish	– –
6.4	++++ ++++	Olive-green " "	+ +	6.4	++ Contam.	Buff	–
7.0	+++ Contam.	Olive-green	+	7.0	++ ++	Buff "	– –
7.6	+++ +++	Blue-green " "	+ +	7.6	Contam. Contam.		
8.4	+++ Contam.	Blue-green	+	8.4	++++ ++++	Dark-gray " "	– –

TABLE XI (*Continued*)

Coons' Medium

Parent Form				Variant Form			
pH	Growth	Color	Spores	pH	Growth	Color	Spores
2.4	− +	Grayish	+	2.4	++++ ++++	Greenish "	− −
2.8	+ +	Dark greenish Dark green	+ +	2.8	++++ ++++	Grayish-black " "	− −
3.2	++++ ++++	Light buff " "	+ +	3.2	++++ ++++	Grayish-black " "	− −
3.8	+++ +++	Dark purple " " XX	+ +	3.8	++ ++	Light vinaceous-lilac Light vinaceous-lilac	− −
4.2	+++ +++	Dark purple " " X	+ +	4.2	++ +	Light vinaceous-lilac White, orig. inoc. buff	− −
5.0	+++ +++	" " XX " "	+ +	5.0	++ +++	Pale vinac. " "	− −
5.6	+++ +++	" " " "	+ +	5.6	+++ Contam.	" "	−
6.4	++++ ++++	Purple X Purplish-brown	+ +	6.4	++++ +++	Light vinaceous-lilac Buff to creamy " " "	− −
7.0	++++ ++++	Purplish-brown X " "	+ +	7.0	++++ Contam.	Deep vinaceous-lavender	−
7.6	++++ ++++	" " X " " XX	+ +	7.6	++ ++	Deep vinaceous-purple " " "	− −
8.4	+ +	Purplish " XXX	+ +	8.4	++ Contam.	" " "	−

X — White island

TABLE XII

COMPARISON OF THE TWO STRAINS OF *COLLETOTRICHUM LINDEMUTHIANUM*, STRAIN II, WHEN GROWN ON COONS' MEDIUM OF VARYING CONCENTRATIONS: CULTURES IN TEST TUBES

PARENT FORM					VARIANT FORM				
Initial pH	Relative growth after 1½ mos.	Color	Acervuli with spores	pH after 1½ mos.	Initial pH	Relative growth after 1½ mos.	Color	Acervuli with spores	pH after 1½ mos.
2.4	0			2.4	2.4	0			2.4
	0			2.4		0			2.4
2.8		Black			2.8		White *	0	3.2
	+++		+++	2.8		+		0	3.2
3.2	+++	Black	+++	3.6	3.2	+	White *	0	3.6
	+++	"	+++	3.6		+	White *	0	3.5
3.8	+++	"	+++	4.4	3.8	−		−	−
	+++	"	+++	4.6		++	White *	0	4.6
4.2	+++	"	+++	4.6	4.2	++	White *	0	4.6
	+++	"	+++	4.6		++	White *	0	4.6
5.0	+++	"	+++	5.4	5.0	+	White *	0	4.4
	0		0	5.0		+++	White *	0	5.2
5.6	0		0	5.5	5.6	+	White *	0	4.8
	+++	Black	+++	6.6		+++	White *	0	5.4
6.4	+++	"	+++	6.2	6.4	++	White *	0	6.
	+++	"	+++	6.2		−			.
7.0					7.0				
7.6					7.6				
8.4	+++	Black	+++	7.6	8.4				
	+++		+++	7.2		++	White *	0	7.2

* Submerged

TABLE XIII

COMPARISON OF THE TWO STRAINS OF *COLLETOTRICHUM LINDEMUTHIANUM* WHEN GROWN ON RICHARDS' MEDIUM OF VARYING HYDROGEN ION CONCENTRATIONS: CULTURES IN TEST TUBES

PARENT FORM					VARIANT FORM				
Initial pH	Relative growth after 1½ mos.	Color	Acervuli with spores	pH after 1½ mos.	Initial pH	Relative growth after 1½ mos.	Color	Acervuli with spores	pH after 1½ mos.
2.	0				2.	±		0	
	0					0		0	
2.8	+	Not recorded	0	2.6	2.8	+		0	2.8
	0			2.6		+		0	2.8
3.7	+++	Cream	+	4.4	3.7	++	White *	0	4.4
	+++	"	+	5.8		++	White	0	5.1
4.8	++	White *	0	4.8	4.8	−			
	+++	Shell-pink	+	5.7		+++	White *	0	5.2
5.0	++	White *	+	4.8	5.0	++	White *	0	4.6
	+++	Shell-pink	+++	6.6		+++	"	0	3.6
5.8	+++	Shell-pink	++	6.4	5.8	++	White *	0	4.6
	+++	Shell-pink	+++	6.4					
6.4	+++	Shell-pink, but blk. with acervuli	+++	6.3	6.4	++	White *	0	5.4
	+++	" "	+++	6.3		++	"	0	5.0
7.0	+++	" "	+++	6.2	7.0	++	"	0	5.8
	+++	" "	+++	6.4		+++	"	0	4.9
7.6	++	Shell-pink	+	5.9	7.6		"		
	++	"	0	6.0		++	White *	0	5.8
8.4	++	Shell-pink	+++	6.6	8.4	+	"	0	6.5
	++	" "	+++	6.2		+	"	0	6.2

* Submerged

TABLE XIV

COMPARISON OF TWO STRAINS OF *COLLETOTRICHUM LINDEMUTHIANUM*, STRAIN II, WHEN GROWN ON RICHARDS' AND COONS' MEDIA OF VARYING HYDROGEN ION CONCENTRATION: CULTURES IN PREPARATION DISHES WITH FILTER PAPER CONES

RICHARDS' SOLUTION							
PARENT FORM				VARIANT FORM			
pH	Growth	Color	Spores	pH	Growth	Color	Spores
2.	– –			2.	– –		
2.8	+++ +++	Black "	+ +	2.8	– –		
3.7	++++ ++++	" "	+ +	3.7	Contam. "		
4.8	++++ ++++	" "	+ +	4.8	Contam. +		
5.	Contam. ++++	 Black	 +	5.	Contam. "		
5.8	++++ Contam.	"	+	5.8	Contam. +	 Creamy	 –
6.4	++++ ++++	Black "	– –	6.4	++++ Contam.	Cream	–
7.	++++ Contam.	"	–	7.	Contam. ++++	 Cream	 –
7.6	++++ ++++	" "	+ +	7.6	+++ +++	" "	– –
8.4	++++ Contam.	"	+	8.4	+++ +++	Cream "	– –

TABLE XIV (*Continued*)

COONS' SYNTHETIC SOLUTION

PARENT FORM				VARIANT FORM			
pH	Growth	Color	Spores	pH	Growth	Color	Spores
2.4	+ Contam.		−	2.4	+ +	Cream "	− −
2.8	++ ++++	Greenish-black " "	− +	2.8	++++ ++++	Dark gray " "	− −
3.2	++++ ++++	Black "	+ +	3.2	++++ ++++	Grayish-black Jet black in places	− −
3.8	++++ ++++	" "	+ +	3.8	++++ ++++	Jet black in places " "	− −
4.2	++++ ++++	Black "	+ +	4.2	+++ ++++	Red-brown Black	− −
5.	++++ ++++	" "	+ +	5.	++ ++	Black "	− −
5.6	+++++ +++++	" "	+ +	5.6 5.	++ ++	" "	− −
6.4	++++ ++++	Black "	+ +	6.4	+ Contam.	Black	−
7.	++++ ++++	" "	+ +	7.	+++ +++	Black "	− −
7.6	++++ ++++	" "	+ +	7.6	+++ Contam.	"	−
8.4	++++ ++++	" "	++ ++	8.4	++ ++	Black "	− −

TABLE XV

EFFECT OF TEMPERATURE AND REACTION ON COLOR AND GROWTH ON THE PARENT AND VARIANT STRAINS OF *CLADOSPORIUM FULVUM* GROWN ON MODIFIED SHIVE'S BEST MEDIUM OF VARYING pH AND KEPT AT DIFFERENT TEMPERATURES

TEMPERATURE 30–32° C.

No growth beyond the point of inoculation at which a slight weak growth was observed 54 days after the inoculation

TEMPERATURE 10° C.

No growth whatever

TEMPERATURE 20–25° C. After 3 days			After 8 days	
	Diam. mm.		Diam. mm.	
pH 4.0				
Parent	3.0	Slight growth, whitish	6.5	Surf. Light olive-green Under. Red-brown
Variant	2.5	White	5.5	Surf. White Under. Yellow-brown
pH 5.0				
Parent	1.5	Greenish-brown	4.5	Surf. Light olive-green Under. Green
Variant	2.0	White	4.0	Surf. White Under. Dark yellow-brown
pH 6.0				
Parent	2.0	Greenish-brown	3.0	Surf. Light olive-green Under. Green
Variant	3.0	White	4.5	Surf. White Under. Dark yellow-brown
pH 7.0				
Parent	2.0	Greenish-brown	6.0	Surf. Light olive-green Under. Purple-brown
Variant	2.5	White	4.5	Surf. White Under. Orange-brown
pH 8.0				
Parent	2.5	Greenish-brown	5.5	Surf. Light olive-green Under. Purple, white edge
Variant	3.0	White	5.0	Surf. White Under. Purple

TABLE XV (*Continued*)

TEMPERATURE 20–25° C. After 54 days

	Diam. mm.	
pH 4.0		
Parent	10	Surf. Brownish-olive 30
		Under. Dull greenish-black 47
Variant	20	Surf. White center, clay color edge
		Under. Hessian brown center, ochraceous-orange edge
pH 5.0		
Parent	9	Surf. Light brownish-olive 30
		Under. Dull greenish-black 47
Variant	20	Surf. Pale yellow-orange
		Under. Hazel 14
pH 6.0		
Parent	5	Surf. Light brownish-olive 30
		Under. Dull greenish-black 47
Variant	15	Surf. White center, ochraceous-buff edge
		Under. Hazel 14
pH 7.0		
Parent	12	Surf. Light brownish-olive 30
		Under. Dull greenish-black 47
Variant	7	Surf. White-light ochraceous-buff
		Under. Hessian brown
pH 8.0		
Parent	6	Surf. Light brownish-olive 30
		Under. Olivaceous greenish-black 47
Variant	12	Surf. White-pale ochraceous-buff
		Under. Hessian brown

EXPLANATIONS OF PLATES

PLATE VIII

FIG. 1. *Septoria apii* with "white island" (left)
Cladosporium fulvum with "white island" (right)

FIG. 2. *Colletotrichum lindemuthianum*, normal culture (right), contrasted with culture in which white variant is developing

PLATE IX

Cladosporium fulvum. Parent form, *A;* variant form, *B* (Camera lucida drawings)

PLATE X

Septoria apii growing upon various combinations of nutrients

PLATE XI

Colletotrichum lindemuthianum, Strain I, growing upon various combinations of nutrients

PLATE XII

Colletotrichum lindemuthianum, Strain II, growing upon various combinations of nutrients

PLATE XIII

Colletotrichum lindemuthianum grown in liquid media (various combinations) to determine relative growth (cf. Table IV)

PLATE XIV

Cladosporium fulvum, parent form, growing on various combinations of nutrients. "White islands" appearing in certain cultures. Grown in light

PLATE XV

Cladosporium fulvum, parent form. Same combinations as in Plate XIV, but grown in dark

PLATE XVI

Cladosporium fulvum, variant form, growing on various combinations of nutrients. Grown in light

PLATE XVII

Same as Plate XVI, but grown in dark

PLATE XVIII

FIG. 1. Tomato leaves (*a*, *b*) inoculated with parent form of *Cladosporium fulvum.* Check leaf at *c.*

FIG. 2. Tomato leaves (*a*, *b*) inoculated with variant form of *Cladosporium fulvum.* Check leaf at *c.*

PLATE VIII

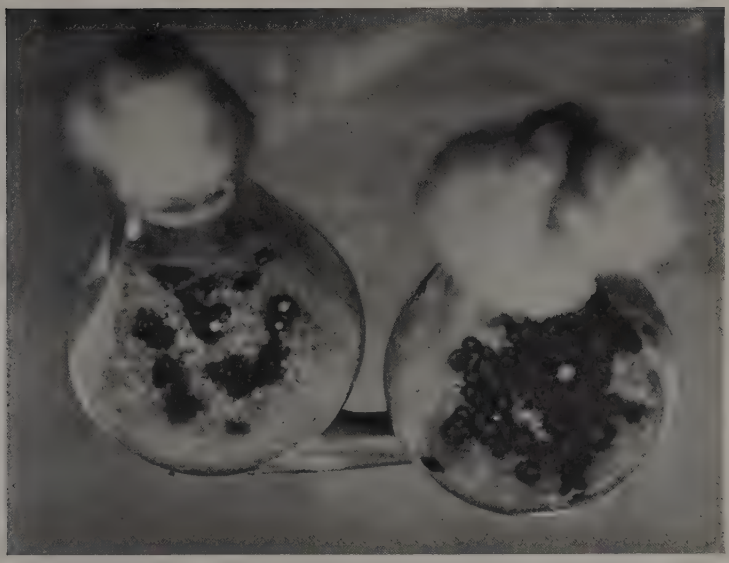


FIG. 1

FIG. 2

PLATE IX

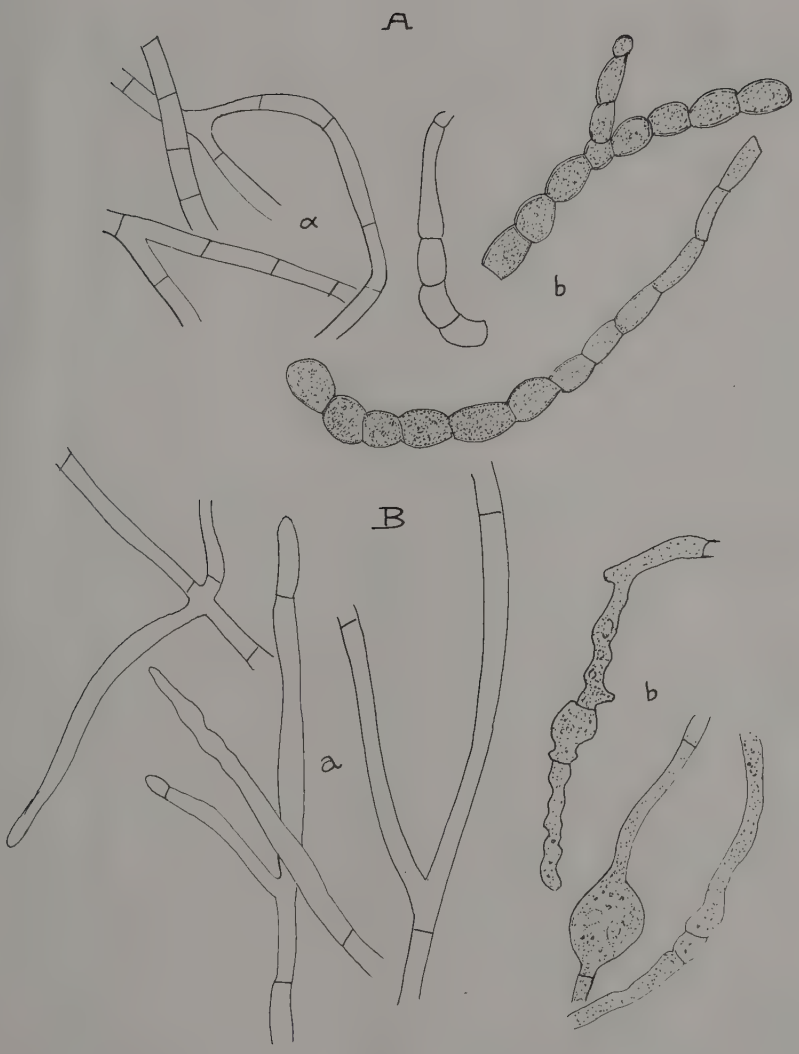


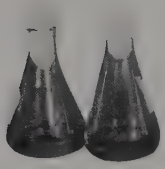
PLATE X

PLATE XI

PLATE XII

PLATE XIII

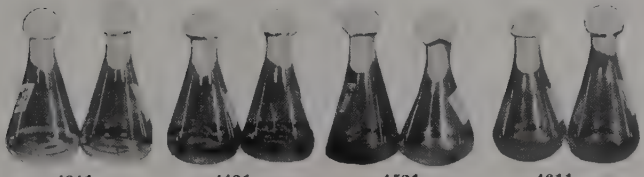
Maltose



7311

6321 6411

5331 5421 5511



4341 4431 4521 4611

3351 3441 3531 3621 3711

Asparagin KH_2PO_4

PLATE XIV

PLATE XV

PLATE XVI

PLATE XVII

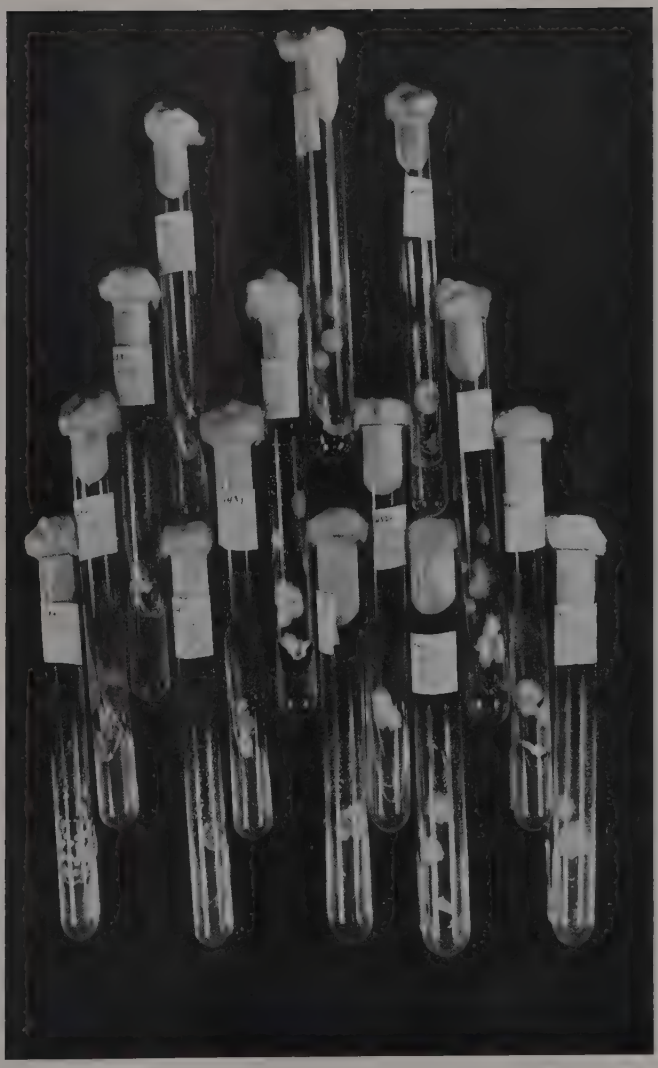


PLATE XVIII

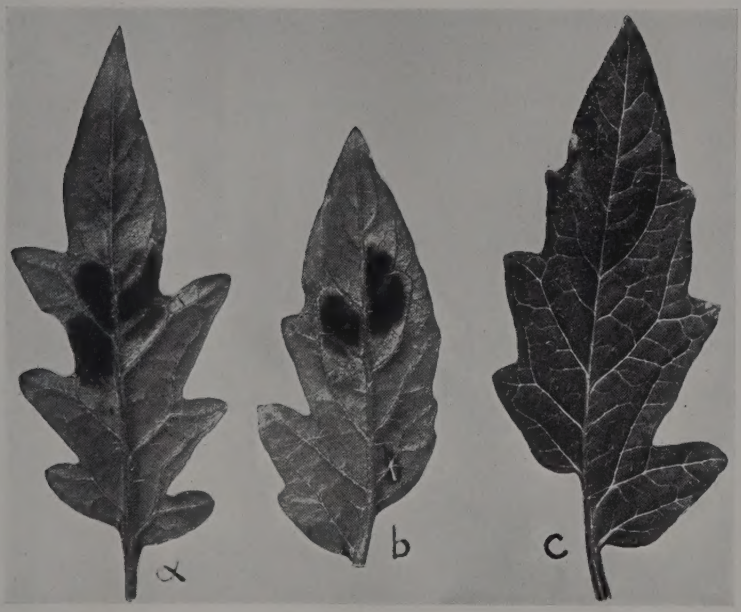


FIG. 1

FIG. 2